

HISTOLOGICAL CHANGES IN THE ANTENNA OF *TENEBRIO*
MOLITOR L. AFTER IMAGINAL ECLOSION
(COLEOPTERA: TENEBRIONIDAE)

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Abstract.—The histology of the antenna of *T. molitor* and the changes which take place in its maturation from imaginal eclosion to one week of age are compared. The cuticle, epidermis, nerves and tracheae are described. A mesocuticle is present in the cuticle, and a blood vessel is described for the first time in the antenna of a beetle. In the one week old insect the endocuticle becomes thicker, the underlying epithelial cells lower in height, the layer of nerve fibers beneath the epithelium more compact, and the walls of the tracheal trunks and blood vessel thinner.

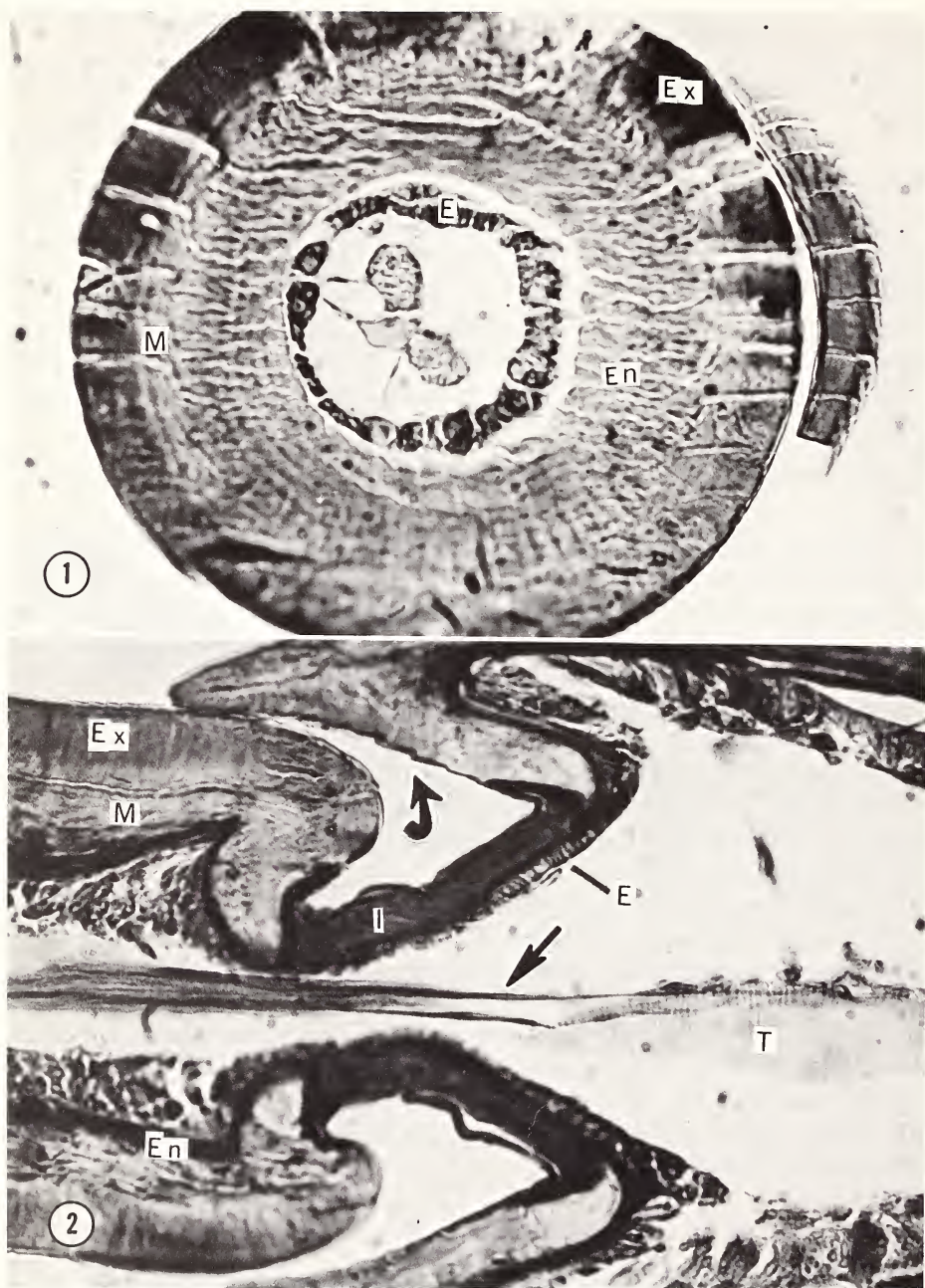
Only two studies include observations on the anatomy of the coleopteran antenna: Borden (1968) on the antennae of the scolytid *Ips confusus* and Chang and Jensen (1973) on the curculionid *Rhabdocelus obscurus*. Most investigations of this organ have concentrated on the morphology and physiology of its sensilla (Roth and Willis 1951; Slifer and Sekhon 1964; Moeck 1968; Ernst 1969; and Borg and Morris 1971). The sensory receptors of *T. molitor* have been studied by Valentine (1931), Pielou (1940), Doyen (1966), Pierantoni (1974), and Harbach and Larsen (1977a, b).

This paper is the first description of the histology of the antenna of *T. molitor* and describes the changes which take place during the first week after imaginal eclosion.

Newly emerged and one week old adults were removed from individual cultures and immediately placed in alcoholic Bouin's solution where the antennae with their musculature were removed. After fixation for 12 hours, they were washed and stored in 80% ethyl alcohol. The antennae were processed and sectioned by a technique previously described (Trombetta and Forbes 1977). The sections were stained with Harris' haematoxylin counterstained with eosin Y, Sharp's modification of Mallory's triple stain (Kennedy 1932), safranin counterstained with fast green, and an argentaffin staining technique (Lillie 1954).

Observations

One week old adult. The antenna of *T. molitor* is moniliform in shape and consists of the scape, the pedicel, and a flagellum that has nine subsegments or annuli joined to each other by membranes. These annuli give the flagellum



Abbreviations: BV—blood vessel, E—epidermis, En—endocuticle, Ex—exocuticle, I—intersegmental membrane, J—sensillum of Johnston's organ, M—mesocuticle, N—nerve trunk, S—sense cell, Sn—sensory receptor, T—trachea.

a segmented appearance, but, because they do not contain muscles, are not true segments (Schneider 1964). Longitudinal sections of the antennae show a segmental arrangement of the annuli, and in recording the observations the older description of the antenna, that it consists of 11 segments, is used. The procuticle of the adult antenna consists of three layers: the exocuticle, the mesocuticle and the endocuticle (Fig. 1). The exocuticle is heavily sclerotized, and in sections it is amber in color and does not readily stain. The thickness of this layer varies in each segment from $23\ \mu$ at the base to $17\ \mu$ at its terminal end. The only exception to this is in the distal segments where because of the numerous sensory receptors it can be as thin as $9\ \mu$. In the intersegmental regions the proximal segment forms a depressed ring into which the base of the next distal segment fits. On the inner surface of the ring, the exterior of the exocuticle is serrated (Fig. 2).

A mesocuticle is present beneath the exocuticle from the scape to segment ten. This layer varies in thickness, stains with safranin and eosin, and except for some red blotches does not stain with Mallory's stain (Figs. 1 and 2). No mesocuticle is seen in the eleventh segment.

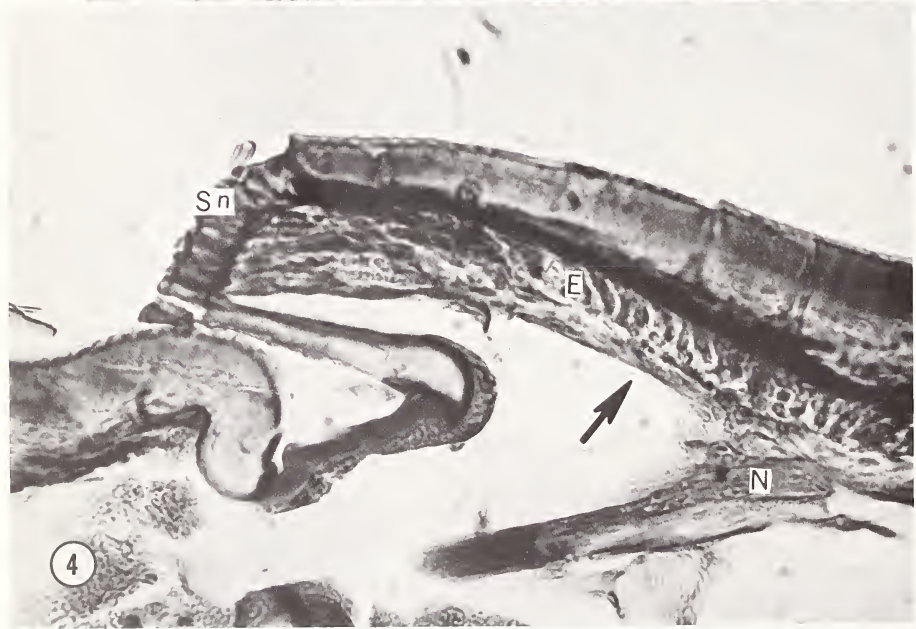
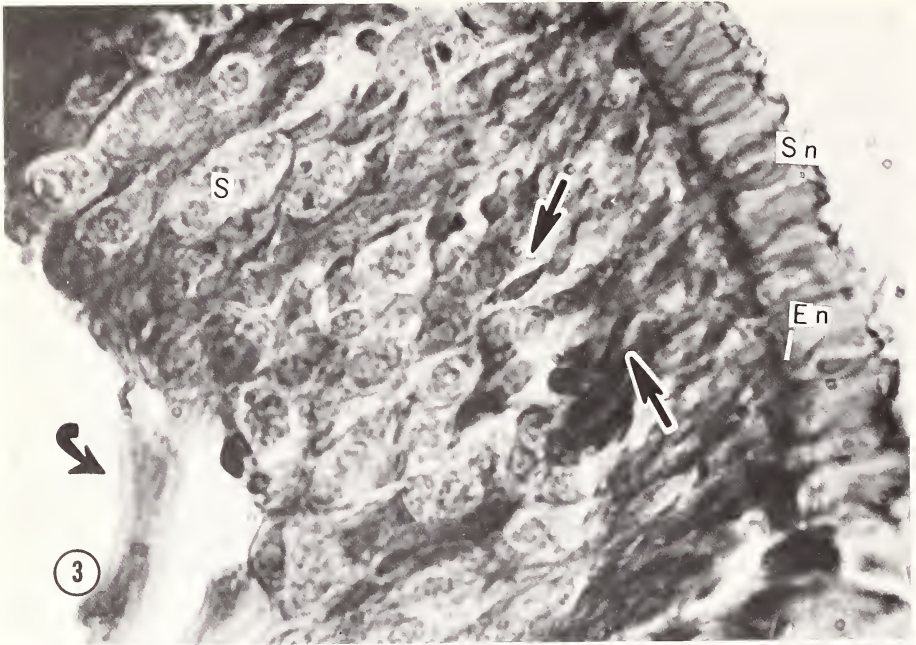
The endocuticle lies beneath the mesocuticle, except in the eleventh segment where it lies directly beneath the exocuticle. It stains lightly with haematoxylin and with fast green and red with Mallory's stain. This layer is laminated and is thickest at the bases of segments six to eleven (Fig. 1). Here as many as ten lamellae are visible, and it is twice as thick as the exocuticle. In the remaining segments the thickness of the endocuticle varies, but generally it is thickest in the center of the segment and is thinnest at the articular regions; slightly thicker at the proximal than at the distal. Where numerous sensory receptors are located, the endocuticle appears as a very thin layer, about $3\ \mu$ in thickness (Fig. 3). In other regions the dendrites of nerves traverse the thick endocuticle to innervate receptors. In the scape the endocuticle is thin and never exceeds five lamellae in thickness.

Pore canals appear under the oil immersion lens as very fine striations extending through the endo-, meso-, and exocuticle. When stained with the argentaffin staining technique, they appear tubular in structure, and are straight, not helical, and end in a small bulb beneath the epicuticle.

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Fig. 1. Cross section of a one week old adult antenna showing the arrangement of the cuticular layers and the epidermis. Haematoxylin and eosin. $\times 400$.

Fig. 2. Longitudinal section through the intersegmental membrane between two antennal segments of a one week old adult antenna. The endocuticle appears dark because it is stained red with Mallory's. Note the serrated edge on the depressed ring of the proximal segment (curved arrow). The blood vessel appears as a smooth-surfaced tube (straight arrow). Mallory's stain. $\times 400$.



The intersegmental membrane between the antennal segments is a thin, unsclerotized membrane, which appears white in color when unstained (Fig. 2). It stains with fast green and appears as a single layer continuous with the endocuticle of the adjoining segments. Mallory's stains two layers of the intersegmental membrane, an exterior layer which stains red and the interior layer which stains blue. In some of these intersegmental membranes random areas of the interior layer have an amber color not unlike that of the exocuticle.

The epidermis underlying the cuticle is composed of a simple epithelium (Fig. 1) that varies from columnar to squamous depending on location and density of the nervous tissue. In the proximal segments, where the sensory receptors and the underlying nerve fibers are not numerous, the cells are cuboidal or columnar. In the distal segments where these nervous elements are numerous the cells are lower in height and are scattered among the sensory cells. At the bases of the distal segments the cells are cuboidal and appear small in comparison to the overlying cuticle. In the intersegmental membranes the cells are also cuboidal. These epidermal cells have a large, centrally located nucleus with peripheral chromatin, and their cytoplasm is extremely basophilic in staining reaction. These cells stain strongly with safranin and with Mallory's stain they appear brown yellow in the proximal segments but brown red in the distal.

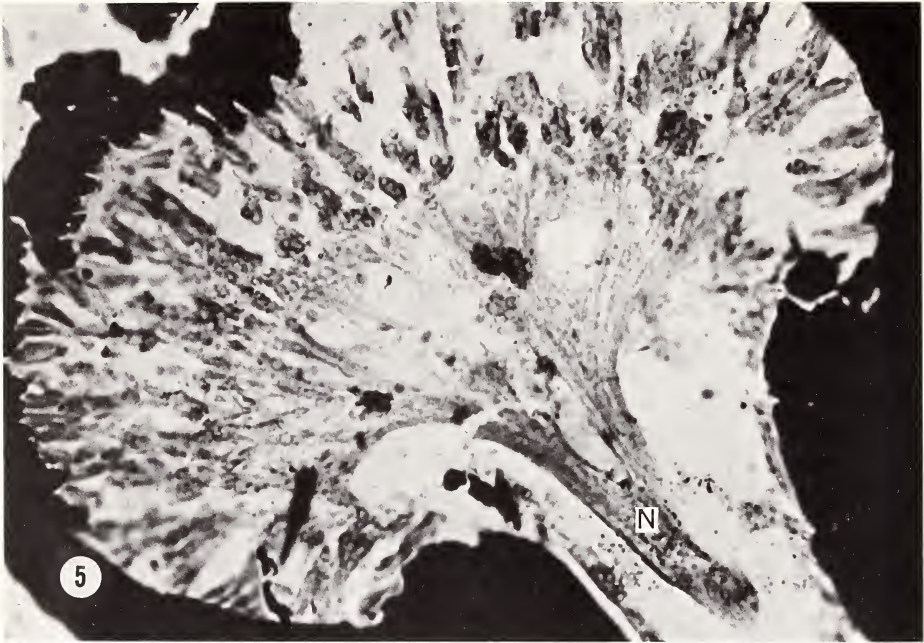
In some regions of the antenna, between the epidermal cells and the endocuticle a very thin, blue green layer can be seen in sections stained with Mallory's stain. Beneath the epidermis the basement membrane is seen as a fine, blue green layer when stained with Mallory's stain. However, when nerve fibers and fine tracheae underlie the epidermal cells, the basement membrane is not evident.

The sensory cells of the adult antenna are typical for insects. They have short, distal dendrites and long axons that join to form large branches. The axons and dendrites stain with fast green and stain light blue with Mallory's stain. The cytoplasm of the cell body of these cells stains deep red with safranin and blue with Mallory's stain. The nucleus stains darker than the cytoplasm and contains peripheral chromatin. Sensory cells of the receptors are found either singly or in clusters (Figs. 3, 4) throughout the antenna.

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Fig. 3. Cross section through numerous sensory receptors in a distal segment of a one week old adult antenna. Note the elongated and scattered epidermal cells (straight arrows) and a nerve branching to the sense cells (curved arrow). The endocuticle appears as a thin layer. Mallory's stain. $\times 1,000$.

Fig. 4. Longitudinal section through a distal segment of a one week old adult antenna showing a branch of the nerve trunk (straight arrow) extending toward an aggregation of sensory receptors. Mallory's stain. $\times 400$.



A large antennal nerve extends from the deutocerebrum of the brain to the antenna. In the scape it innervates the intrinsic antennal muscles and then divides to form two nerve trunks that extend up the antenna and end in terminal arborizations in the eleventh segment. These nerve trunks give off many fine branches which innervate the solitary sensory receptors, while they extend up toward the distal segments. In these distal segments where there are aggregations of sensory receptors, larger nerve branches split from the antennal nerve trunks and innervate them (Figs. 4, 5). Each nerve trunk is surrounded by a thin nerve sheath with flat nuclei (Fig. 6). Sections of the nerve trunks show an intense safranin staining central area, that stains darker than the peripheral nerve sheath nuclei.

Johnston's organ can be seen in cross sections of the pedicel as clusters of sensory cells encircling the nerve trunks, the blood vessel and the tracheae (Fig. 7). These clusters of sensory cells are attached by their axons to branches of the nerve trunks, and their dendrites extend into the inter-segmental membrane between the pedicel and third segment (Fig. 8).

The nervous tissue in the antenna of the one week old adult is associated with the epidermis in three ways. First, sensory cells may lie next to epidermal cells with their axonal fibers extending to an antennal nerve branch as in the case of a solitary receptor or a small aggregation of receptors (Fig. 3). Second, a nervous layer lies immediately beneath the epidermal cells. This layer consists of nerve branches extending from the nerve trunks and from which dendrites extend to the receptors (Fig. 4). Third, nerves have almost completely displaced the epidermal cells as in the eleventh segment.

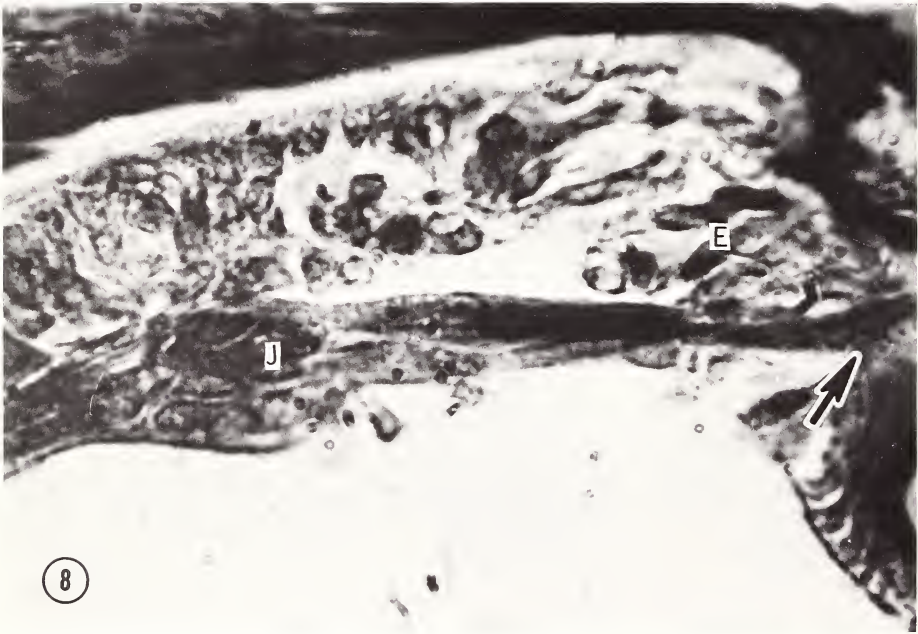
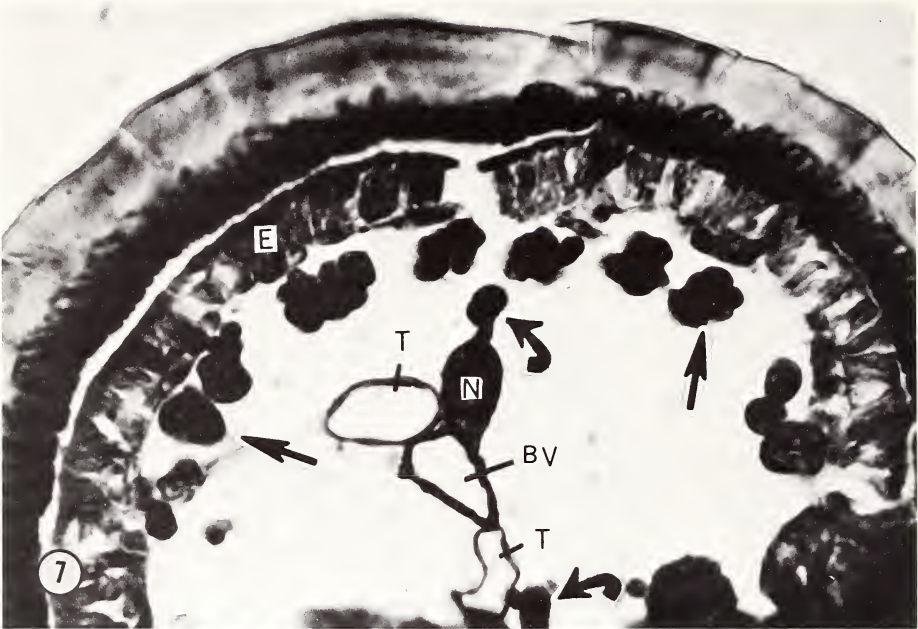
A large trachea enters the antenna, and in the third segment it bifurcates. These two tracheae course through the lumen of the antenna and send tracheoles to the epidermal cells and to the sensory cells (Figs. 6, 7). The tracheoles are extremely numerous in regions where there are numerous receptors. They vary in size and some of the smallest lie against the basement membrane of the epidermis. They give the appearance of being rigid and circular in shape or collapsed. The tracheae are composed of extremely thin cells with few nuclei and these protrude into the antennal lumen. Internal to this epidermal lining a very thin cuticle with taenidia surrounds the tracheal lumen (Figs. 2, 6).

The blood vessel is found among the nerve trunks and tracheae and extends straight through the antenna with no branches. It is always closely

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Fig. 5. Oblique section through the last antennal segment of a one week old adult antenna showing the arborization of an antennal nerve. Argentaffin stain. $\times 80$.

Fig. 6. High power view of a cross section of a one week old adult antenna showing the arrangement of nerve trunks, tracheae and blood vessel. Arrows indicate the nuclei of the nerve sheath and tracheae. Haematoxylin and eosin. $\times 1,400$.



associated with one of the nerve trunks and usually lies directly against it. The diameter of the blood vessel varies throughout the antenna, but it is always largest in the third segment. No pores or openings in the vessel were seen, not even at the tip. The wall of the blood vessel has many nuclei and generally appears thicker than that of the tracheae.

Newly emerged adult.—The antenna of the newly emerged adult is not heavily sclerotized. The exocuticle ranges in thickness from $23\ \mu$ at the bases of the antennal segments to $17\ \mu$ in the terminal regions of the proximal segments and $14\ \mu$ in the terminal regions of the distal segments. This layer is similar to that of the one week old adult in that it is thinnest where there are aggregations of sensory receptors. In unstained sections its color is light yellow in the proximal segments and amber in the distal regions (Figs. 9, 10). Only when the antenna is stained with Mallory's stain can light red blotches be seen in the exocuticle. The mesocuticle appears as a very thin layer throughout the antenna when stained with safranin and eosin but not with Mallory's stain (Figs. 9, 10). The endocuticle ranges in thickness from $2\ \mu$ to $3\ \mu$. It is thickest at the bases of the segments as well as in the terminal regions of the proximal segments. It is present as a very thin layer in areas of the antenna where there are aggregates of sensory receptors. It stains with haematoxylin, fast green, and red with Mallory's stain. Where epidermal cells have pulled away from the endocuticle in sections of the newly emerged adult, its inner edge is highly irregular but fits into the surface contours of these cells. When this artifact is seen in sections of the one week old adult beetle, the inner edge of the endocuticle is relatively smooth. The pore canals are present and are similar in arrangement and position to those in the cuticle of the one week old adult beetle.

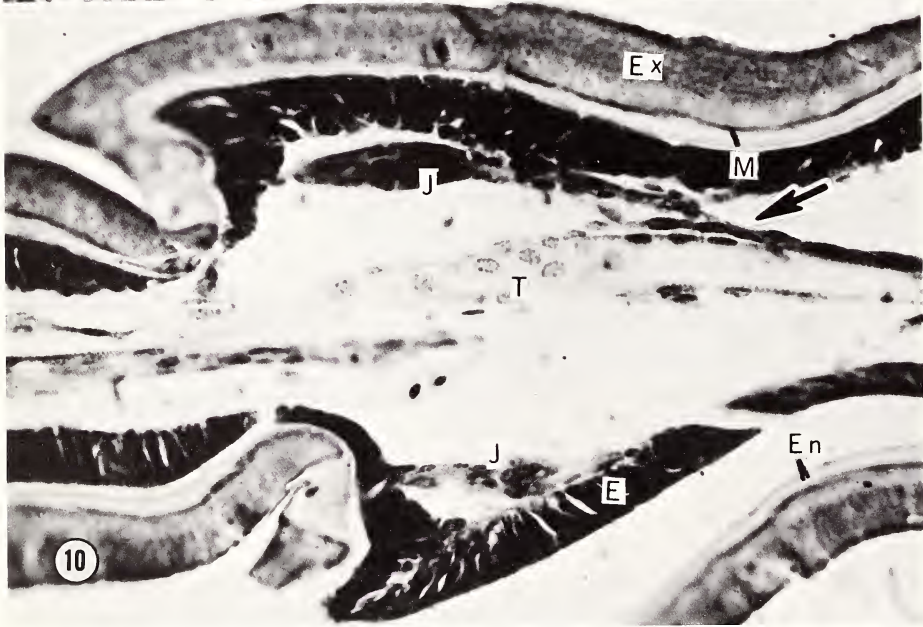
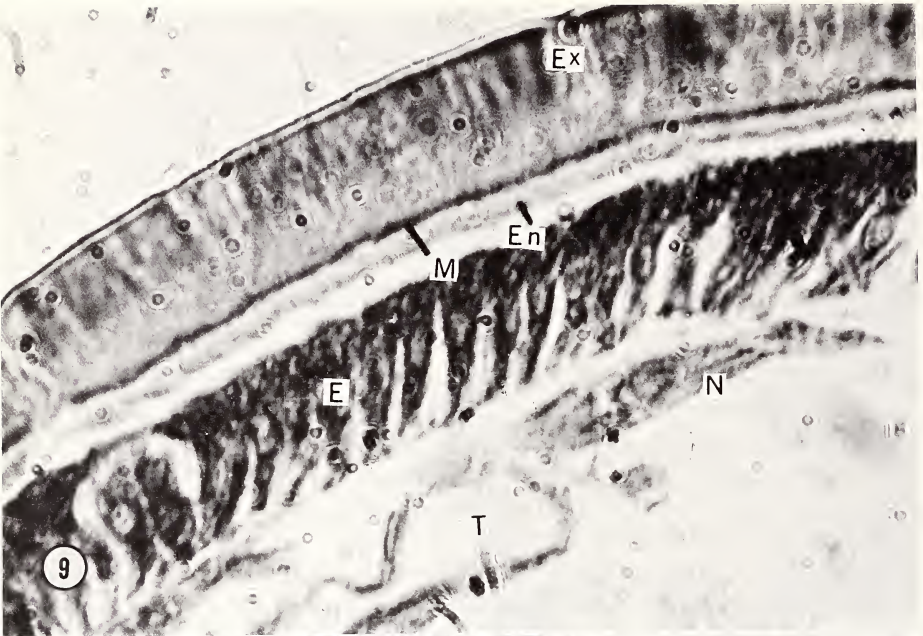
The cuticle of the intersegmental membrane is thinner than that of the segments and consists of two layers. The inner layer, $10\ \mu$ thick, stains blue with Mallory's stain while the outer, only $3\ \mu$ thick, ranges in color from clear, to faint purple, to dark purple. The cuticle of this membrane, when stained with fast green, appears to be a single layer.

The epidermis throughout the antenna is composed of tall columnar cells that narrow slightly at their bases (Figs. 9, 10). In the proximal segments the cells are taller than the thickness of the cuticle, and in the basal regions

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Fig. 7. Cross section of the pedicel of a one week old adult showing the sensory cells of Johnston's organ (straight arrows). Curved arrows point to branches of the nerve trunk. Mallory's stain. $\times 540$.

Fig. 8. Longitudinal section through the pedicel of a one week old adult showing sensory cells of Johnston's organ. Arrow indicates attachment of the fibers of these cells to the intersegmental membrane between the pedicel and the third segment. Mallory's stain. $\times 1,000$.



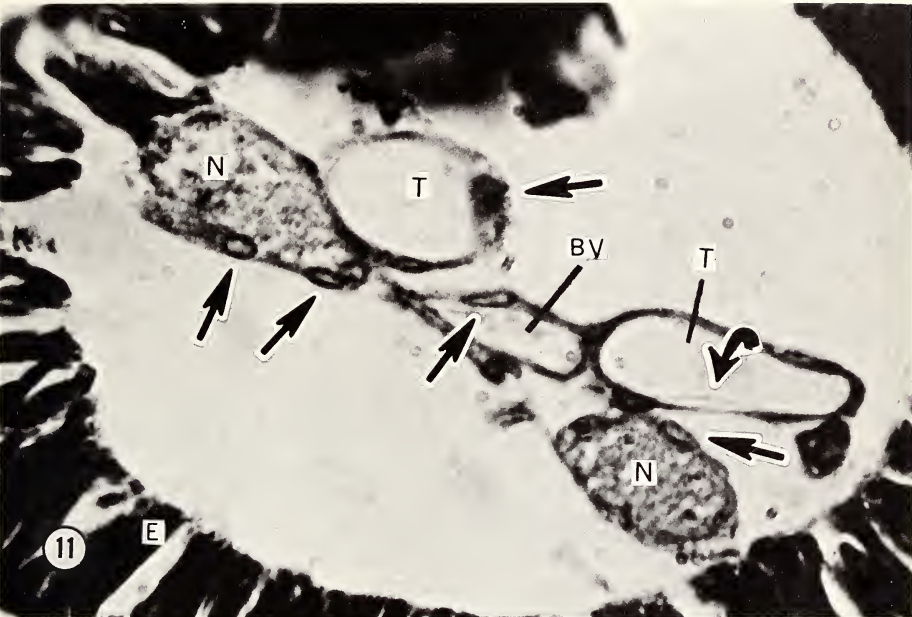


Fig. 11. High power view of a cross section of the antenna of a newly emerged adult showing the arrangement of the nerve trunks, tracheae and blood vessel. Straight arrows indicate nuclei of these various structures and the curved arrow the cuticle of the trachea. Haematoxylin and eosin. $\times 1,000$.

of the distal segments these cells are as tall as the thickness of the cuticle. In the terminal region of the distal segments they are taller than the cuticle. The epidermis is never displaced by nervous tissue even in areas where there are numerous sensory cells. These cells have a large, centrally located nucleus that has both peripheral chromatin and large, round, dense chromatin granules scattered throughout the karyoplasm. The cells stain intensely with safranin and brown yellow to brown red with Mallory's stain. The cytoplasm close to the endocuticle is filled with basophilic granules which also stain faintly blue with Mallory's stain. A very thin basement membrane is visible, which stains faintly blue green with Mallory's stain. The epidermal cells of the intersegmental membrane are very small, cuboidal in shape,

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Fig. 9. High power view of a cross section of the antenna of a newly emerged adult showing the arrangement of the cuticular layers and the epidermis. Haematoxylin and eosin. $\times 1,000$.

Fig. 10. Longitudinal section of the pedicel of a newly emerged adult showing the arrangement of the antennal structures and sensory cells of Johnston's organ. The arrow points to a nerve innervating these sensory cells. Haematoxylin and eosin. $\times 400$.

	ONE WEEK OLD	NEWLY EMERGED
EXOCUTICLE	does not stain	generally does not stain but shows some light red blotches with Mallory's stain
MESOCUTICLE	safranin, eosin, red blotches with Mallory's stain	safranin, eosin
ENDOCUTICLE	haematoxylin, fast green, red with Mallory's stain	haematoxylin, fast green, red with Mallory's stain
INTERSEGMENTAL MEMBRANES	fast green, red and blue with Mallory's stain	fast green, blue and purple with Mallory's stain
EPIDERMIS	safranin, brown yellow and brown red with Mallory's stain	safranin, brown yellow and brown red with Mallory's stain
NERVES	soma: red with safranin and blue with Mallory's stain axons and dendrites: fast green, light blue with Mallory's stain	soma, axons and dendrites: safranin, brown yellow and brown red with Mallory's stain

Table 1. Summary of staining characteristics of antennal structures between one week old and newly emerged adults.

and have a large, centrally located nucleus, similar to the other epidermal nuclei. The basophilic area above the nucleus is also present but it is not as clearly defined as in the cells beneath the endocuticle of a segment. The basement membrane is not discernible beneath these cells.

The central body and axons of the sensory cells resemble the bipolar cells of the one week old adult, except that the dendritic processes are thicker and not as long. In the regions where there are aggregations of sensory receptors the dendrites are very short, while the dendrites for the solitary sense receptors are longer and more closely resemble the dendrites of the older beetle. The cuticle at the apex of the receptor surface of the newly emerged adult is not as sharply defined but appears more blunted than in the one week old adult. The nerve trunk (Fig. 11), the fibers, and the sensory cells all stain readily with safranin and with Mallory's stain, similar to the epidermal cells. At this age there is no way to distinguish nerve cells from epidermal cells other than position and shape.

In the newly emerged adult the nerve trunks and branches are more loose in their arrangement; they are not as compact as are those in the older beetle. However, they are well developed, and many fine nerve branches extend to the sensory cells. Johnston's organ is fully developed in the newly emerged adult (Fig. 10).

The tracheae in this stage are arranged in the same manner as are those in the one week adult. Their walls are thicker, the epithelial cells are taller and the nuclei are larger and not as flat as are those of the older beetle. The tracheal cuticle is clearly visible but its inner surface is not as smooth as that of the older beetle (Fig. 11). The wall of the blood vessel is thicker than that of the one week old beetle, and the nuclei do not bulge into the antennal lumen (Fig. 11). Throughout the antennal lumen, but not within the blood vessel, there is a granular substance which stains with fast green and blue with Mallory's stain.

Discussion

This study shows that the procuticle of the antenna of *Tenebrio* has three layers, and that the mesocuticle is deposited before the endocuticle. The variations of staining characteristics of the different cuticular layers (Table 1) indicates changes in chemical composition. Wigglesworth (1948), Delachambre (1967), and Zlotkin and Levinson (1969) have described only two layers, the exocuticle and endocuticle, in the abdomen of *Tenebrio*. The cuticle of the pupal abdomen was described by Delachambre (1967), Zlotkin and Levinson (1969), and Caveney (1970). Both Delachambre and Caveney mention that the procuticle is composed of two layers, and that the endocuticle is completely deposited within twenty-four hours of pupation.

The epidermis of the antenna undergoes extreme changes during its development. After secretion of the adult cuticle, the epidermal cells are reduced in size and their reduction accompanies the increased development of the nervous tissue. The large, dense, chromatin granules of the epidermal cells of the developing antenna have been seen also in the cells of the abdominal cuticle (Zlotkin and Levinson 1968). The basement membrane is never clearly or completely seen in the antenna of *Tenebrio* because tracheae and nerve fibers adhere closely to the epidermis. The basement membrane is similar to that in the antenna of *Bombyx mori* (Schneider and Kaissling 1959).

Johnston's organ in *Tenebrio* is similar to the one described for the beetle, *Melolontha vulgaris* (Child, 1894). Neither Borden (1968) nor Chang and Jensen (1973) mention this organ in the antennae of the beetles they describe.

Except for the changes in cell thickness, the histology of the tracheae found within the antennal lumen of *Tenebrio* is typical for insects. An antennal blood vessel is described for a coleopteran for the first time and appears similar to antennal blood vessels in other insects. The dilated region of the blood vessel in the third segment may represent an accessory pulsating organ. Schneider and Kaissling (1959) describe a blood vessel in the antenna of *Bombyx mori* that had an opening only at its distal end. The lack of any openings in the blood vessel in *Tenebrio* might be explained on the

basis that small pores may be present in the wall which cannot be resolved by the light microscope. The close association of the blood vessel and the antennal nerve trunk suggests either mutual support or that the blood vessel is innervated.

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